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## Article

# Investigating the Geochemical Controls on Pb Bioaccessibility in Urban Agricultural Soils to Inform Sustainable Site Management

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**Abstract:** The solid-phase speciation of contaminants in soil plays a major role in regulating both the environmental mobility of contaminants and their bioavailability in biological receptors such as humans. With the increasing prevalence of urban agriculture, in tandem with growing evidence of the negative health impacts of even low levels of exposure to Pb, there is a pressing need to provide regulators with a relevant evidence base on which to build human health risk assessments and construct sustainable site management plans. We detail how the solid-phase fractionation of Pb from selected urban agricultural soil samples, using sequential extraction, can be utilised to interpret the bioaccessible fraction of Pb and ultimately inform sustainable site management plans. Our sequential extraction data shows that the Pb in our urban soils is primarily associated with Al oxide phases, with the second most important phase associated with either Fe oxyhydroxide or crystalline FeO, and only to a limited extent with Ca carbonates. We interpret the co-presence of a P component with the Al oxide cluster to indicate the soils contain Pb phosphate type minerals, such as plumbogummite ( $\text{PbAl}_3(\text{PO}_4)_2(\text{OH})_5 \cdot \text{H}_2\text{O}$ ), as a consequence of natural “soil aging” processes. The presence of Pb phosphates, in conjunction with our biomonitoring data, which indicates the lack of elevated blood Pb levels in our gardeners compared to their non-gardening neighbours, suggests the (legacy) Pb in these soils has been rendered relatively immobile. This study has given confidence to the local authority regulators, and the gardeners, that these urban gardens can be safe to use, even where soil Pb levels are up to ten times above the UK’s recommended lead screening level. The advice to our urban gardeners, based on our findings, is to carry on gardening but follow recommended good land management and hygiene practices.

**Keywords:** risk assessment; urban gardens; lead; source apportionment

## 1. Introduction

Urban agriculture and community gardening is increasingly endorsed as a health promoting activity, providing a wealth of educational, economic and societal benefits [1,2]. Yet urban soils are frequently reported with high concentrations of a range of potentially toxic elements (PTEs) and gardening in contaminated urban soils has the potential to increase our exposure to PTEs such as Pb [3]. The transfer of Pb to humans can occur via several exposure routes including soil and dust

ingestion, inhalation of particles containing Pb, Pb-contaminated water and through the consumption of food containing Pb [4].

With an increasing body of research highlighting the potential health impacts of even low levels of environmental exposure to Pb [5], there is a growing need to provide greater confidence to regulators, who must decide if sites are suitable for use as urban agricultural sites (UAS), in addition to the general gardening community. To address this need, the Newcastle Allotments Biomonitoring Study (NABS) was initiated in 2014. NABS is a community-research partnership involving the UAS gardeners, the local city council, and researchers across a range of academic and government-funded agencies. As part of this study we undertook the paired sampling of soils and crops, and determined the blood Pb levels (BLL) of both gardeners ( $n = 43$ ) and their non-gardening neighbours ( $n = 29$ ) [4]. We observed no statistically significant difference between the BLL of the gardeners and those of their non-gardening neighbours ( $p = 0.569$ ), despite 98% of the 279 sampled soils reporting total Pb concentrations above the UK soil screening guideline for Pb [4].

To pose a human health risk, the Pb taken into the body must be bioavailable (i.e., available for absorption into the systemic circulation) via, for example, the gastrointestinal (GI) tract. In vitro bioaccessibility studies aim to quantify the fraction of a contaminant in soil that is soluble and readily released during passage through the GI tract, e.g., [6]. Although it is now relatively common to determine the oral bioaccessibility of PTEs in soils as part of human health risk assessments, it is less common to accompany these investigations with complementary lines of geochemical evidence, such as detailed solid-phase fractionation and biomonitoring. Such data, however, provide pivotal information to inform both sustainable site management and targeted intervention strategies. Although the specific aim of this research was to understand the geochemical controls on the bioaccessibility of Pb in soils from urban allotment sites, our wider remit was to provide evidence on the need for regulators to consider the role of “aged” or non-labile Pb in UAS. The selected soils were considered typical of urban allotment soils encountered globally on made-ground, in cities with an industrial heritage. Although none of the sampled sites were on formerly industrial land, each has a long history of additions of coal ash from domestic hearths, in addition to other common urban sources of Pb, including legacy atmospheric sources from the burning of fossil fuels and Pb-painted wood. Our specific objectives were to (i) identify the solid-phase fractionation of Pb from selected soil samples using sequential extraction; (ii) identify common geochemical relationships between soils from different sites; and (iii) relate the solid-phase fractionation of Pb results to the bioaccessible fraction of Pb in each soil.

## 2. Materials and Methods

A subset ( $n = 12$ ) of the 279 topsoils collected from around the roots of the crops sampled as part of the NABS (Entwistle et al. 2019 [4]) were used in this current study to elucidate the geochemical control on the soil Pb. Newcastle is the regional capital of NE England (population 280,200; [7]), and the soil subset was selected to cover 12 allotment gardens across three urban agriculture sites (identified herein as sites 1, 2 and 3). The samples are labelled by their site number followed by a number representing a different sample taken from a different location in that specific site, e.g., Sample 2.2 represents the second sample taken from site 2. Methods for pH and organic matter content are detailed in Entwistle et al. 2019 [4], but in brief were based on a soil-deionized water suspension and the Walkley–Black method [8], respectively. The total Pb concentration in the  $<250 \mu\text{m}$  soils was determined by a  $\text{HNO}_3/\text{HF}/\text{HClO}_4$ -based mixed acid attack with a  $\text{HCl}/\text{HNO}_3$  pre-digest followed by ICP-MS analysis. The bioaccessible Pb was previously determined [4] using the Unified BARGE Method (UBM). Lead bioaccessibilities ( $n = 21$ ) ranged from 32 to 76% (geomean of 58.7%), with bioaccessible concentrations ranging from 58 to 705 mg/kg. The UBM was developed by the BARGE group [9], has been validated against an in vivo swine model for As, Cd and Pb [10], and is an International Standard for determining PTE bioaccessibility in soil [11].

The solid-phase fractionation of the test soils was determined using the Chemometric Identification of Substrates and Elements Distributions (CISED) method [12–15].

The CISED method is a non-specific extraction system utilising chemometric data processing to measure the trace element distributions in soils and sediments. The basis of this type of approach is

that no extraction reagent is considered to be totally specific for its target phase and, as a result, a non-specific reagent is used at increasing concentrations. The resulting data set can then be considered in terms of mixtures of different phases. The mixture of the different dissolved phases is then resolved by the use of chemometric approaches, which are based on multivariate self-modelling mixture resolution procedures [16]. A number of assumptions are made when using this type of data processing, which include “that the material under study consists of a mixture of discrete physico-chemical components with distinct major element compositions and that the trace metals of interest are distributed amongst these components” [16]. Another premise is that the physico-chemical components will dissolve to different degrees and as the reagent strength increases each solution will contain differing proportions of each of the components of the test material. The final assumption of the methodology [16] is that within any given physico-chemical component all of the elements are dissolved congruently. The method is a sequential soil extraction using a simple mineral acid matrix.

Soil samples of approximately 2.00 g were sequentially extracted by the addition of 10 mL of an extraction solution (Table 1) which contained an increasing concentration of nitric acid/hydrochloric acid mixture (i.e., from 0 to 5 M). After adding 10 mL of extraction solution, samples were mixed on an end-over-end shaker for 10 min, and the liquid phase was recovered via centrifugation (4350 g for 5 min) and used for analysis; the soil pellet was re-suspended with the appropriate extraction solution. The 7 extraction solutions (1 of deionized water and 6 of aqua-regia using mixtures of analytical reagent grade nitric and hydrochloric acid) are listed in Table 1.

Each extraction solution (7 solutions) was used twice to obtain a total of 14 extracts (10 mL). As highlighted in Table 1, in the last 8 extractions (7 to 14) increasing amounts of H<sub>2</sub>O<sub>2</sub> were added to the extraction solutions to enhance degradation of organic matter and dissolution of Fe–Mn oxides [17].

**Table 1.** Solutions used for the sequential extraction.

Extraction Order	Extractant Concentration	Volume of Extractant (mL)	No of Repeat Extractions	Volume of 30 Vol H <sub>2</sub> O <sub>2</sub> (mL)
1–2	De-ionized water	10	2	0
3–4	0.01M aqua-regia	10	2	0
5–6	0.05M aqua-regia	10	2	0
7–8	0.1M aqua-regia	9.75	2	0.25
9–10	0.5M aqua-regia	9.50	2	0.50
11–12	1.0M aqua-regia	9.25	2	0.75
13–14	5.0M aqua-regia	9.00	2	1.00

Appropriate quality assurance procedures and precautions were followed to ensure the reliability of these data. All experimental reagents used were of analytical reagent grade. Milli-Q water was used throughout the study. The quality assurance/quality control procedures for the determination of bioaccessible Pb utilised the BGS guidance soil (BGS 102), duplicates and blanks, as described by Entwistle et al. [4]. Duplicates, blank extractions (n = 2) and Certified Reference Materials (n = 3; BCR 701, BGS 102, NIST 2711a [18–20]) were included in the determination of total Pb. Lead recovery from the digestion of the reference materials was  $\pm 10\%$ . Lead in the blank digestions was less than the detection limit (0.02 mg kg<sup>-1</sup>), with a repeatability within 10% for the total digestion. Duplicate extraction and analysis of one sample in the CISED extraction was better than 10%.

The major and trace element data obtained from the sequential extracts for each soil was assembled into a data matrix consisting of 14 rows (the extracts) and 26 columns (the elements). The data for each soil was subjected to a previously described [16,21] Self Modelling Mixture Resolution (SMMR) algorithm. This procedure separates the data into geochemically distinct components, which include the chemical composition of each component, the amount of each component in each extract and the fractionation of each element between each of the identified components. The algorithm is programmed in the MATLAB programming language and uses a bootstrap re-sampling approach to provide median and 95th percentile confidence intervals on all the outputs [16]. Statistical analysis of the CISED outputs and plotting was carried out using the R programming language [22].

### 3. Results

The mean pH of the soils across the three UAS was neutral (pH 7.1, pH range 6.7–7.8), while the soil organic matter content indicated percentages ranging from 5.7 to 25%, with a mean of 18%. The total Pb concentration in the soils in this study ranged between 115 and 1131 mg kg<sup>-1</sup>, with a mean of 487 mg kg<sup>-1</sup>. CISED extractable Pb ranged between 75 and 88% of the total digest values, despite the relatively large range across the total concentrations, indicating a similar extractability between soils and sites.

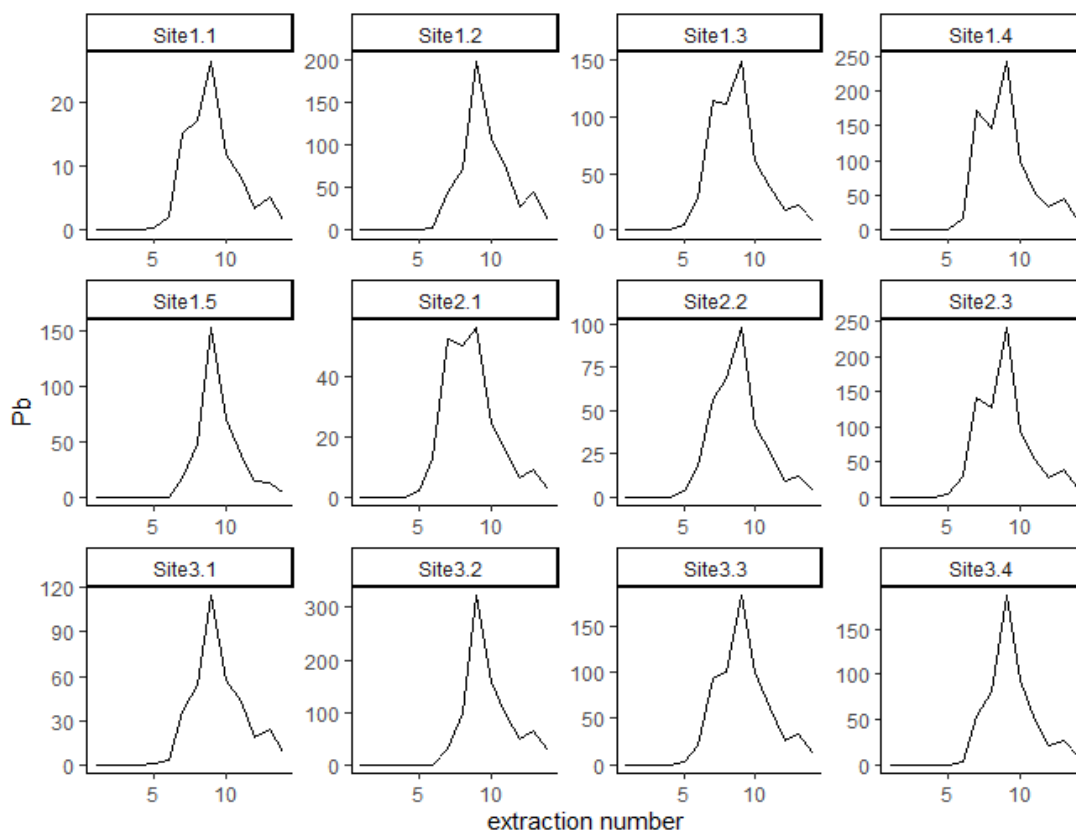
#### 3.1. Pb Fractionation in the Soils

##### 3.1.1. Pb Extraction Profiles

The CISED approach for determining the physico-chemical fractionation of Pb in the test soils has been previously described in a number of studies [13,23–26]. The CISED method is designed to identify the distribution and physical form of PTE within the constituents of any given soil and developed to overcome the problems associated with traditional sequential extraction methods [21]. The advantages of the CISED method over other sequential extraction schemes [27,28] are:

- The method is very fast (ca. 10–15 min per extract);
- The analysis is simple to carry out because there are no reagents with high total dissolved solids (TDS) to cause nebulizer blockages and other analytical problems;
- Rapid extraction and strong acid reagents minimize the potential for elements to be mobilized and redistributed to other phases; and
- The phases identified are a true representation of the natural state of the soil; they are not methodologically defined as in more conventional procedures.

Figure 1 shows the Pb extraction profiles over the 14 CISED extracts for each of the 12 soils studied. The profiles show some similarity between extraction peaks extending from extraction numbers 6 to 14 and with a peak value at extraction number 9–10. This indicates that Pb is extracted at medium to high acid concentration (Table 1).

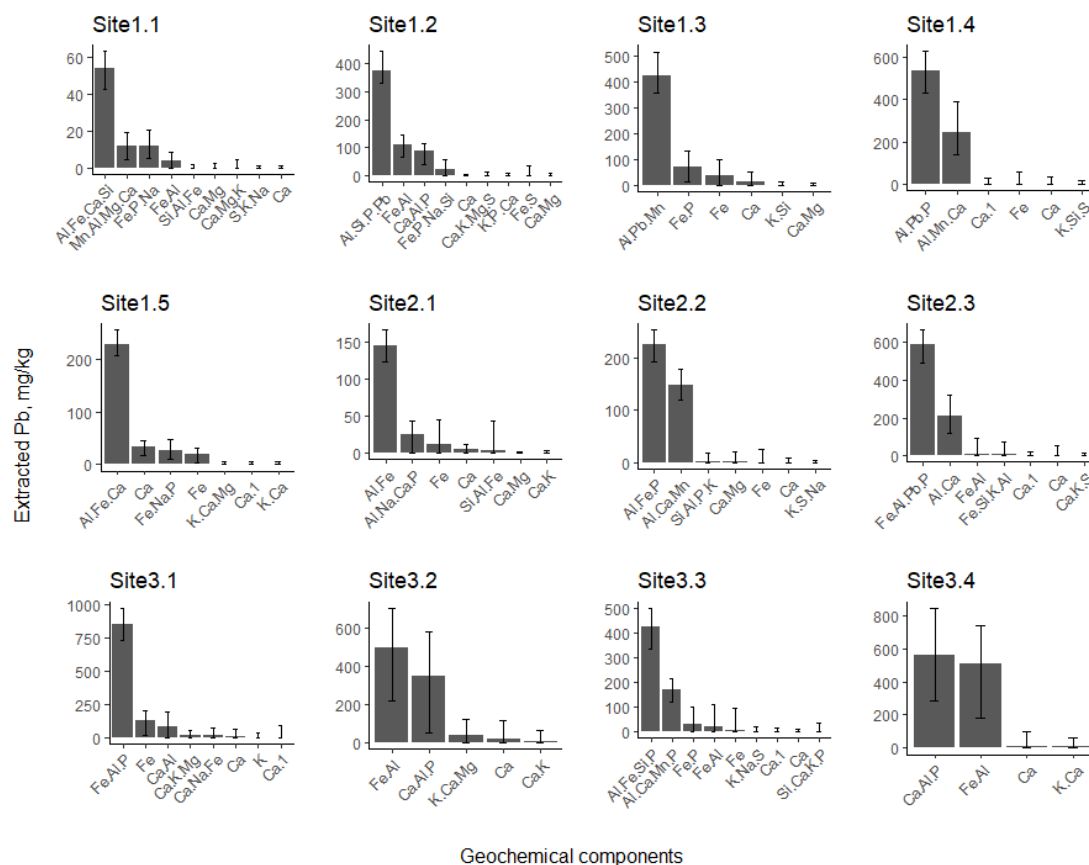


**Figure 1.** Pb extraction profiles,  $\text{mg kg}^{-1}$ .

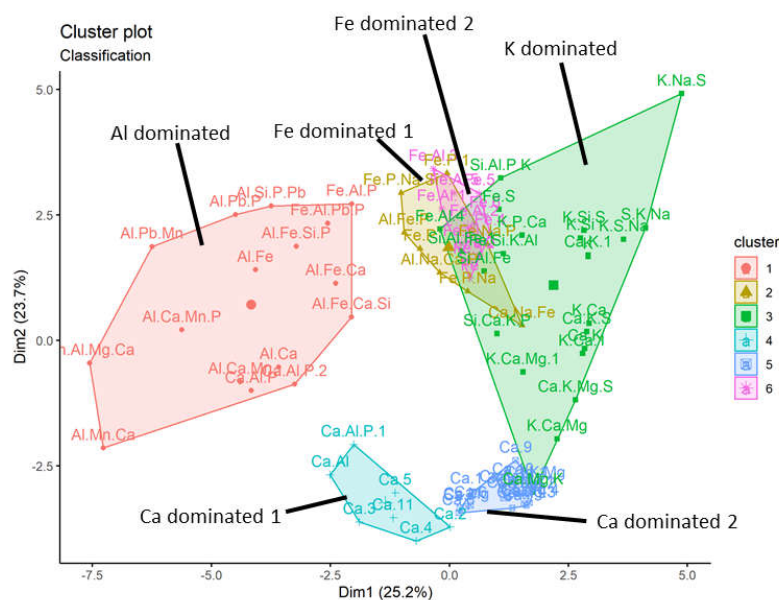
### 3.1.2. Fractionation of Pb Between the CISED Identified Geochemical Components

Figure 2 shows how the Pb in each soil is fractionated between the different geochemical components identified by the CISED extraction. The components are named by the elements that contribute more than 10% by weight of the component composition (in decreasing percent contribution). The components are different for each soil but there are some commonalities between the components in each soil, e.g., Al dominated components, Ca dominated components, Fe dominated components. Components made up predominantly of organic carbon do not have a strong inorganic signature so they are not likely to show up as clearly as the mineral components of the soil. This is not to say that there may be significant amounts of organic matter associated with the components, however Spearman's correlation between the percentage bioaccessibility and the organic matter content indicates there is a significant negative correlation (bootstrapped Spearman correlation coefficient of  $-0.75$  with 95th confidence interval of  $-0.23$  to  $-0.96$ ,  $n = 12$ ). This may indicate that the bioaccessible fraction is more associated with the inorganic fraction of Pb, as suggested by the CISED fractionation. However, this is a relatively small set of samples ( $n = 12$ ) and the controls on Pb bioaccessibility are likely to be a combination of factors [29].

To facilitate direct comparisons between the fractionation of Pb in the samples, a simplified picture of the different geochemical components was obtained by clustering their geochemical compositions into groups based on their element composition. Using a Gaussian mixture modelling algorithm fitted using expectation maximisation (EM) (mclust library of the R programming language [30]) six distinct clusters were identified (Figure 3).



**Figure 2.** Fractionation of Pb between the Chemometric Identification of Substrates and Elements Distributions (CISED) identified geochemical components.



**Figure 3.** Cluster groupings for the geochemical components found in the soil.

Figure 3 shows the six clusters on a principal component analysis (PCA) biplot of the first two principle components. Cluster 1 is an Al dominated cluster, clusters 2 and 6 are Fe dominated clusters, cluster 3 is a K dominated cluster, and clusters 4 and 5 are Ca dominated clusters.

### 3.1.3. Interpretation of the Geochemical Clusters

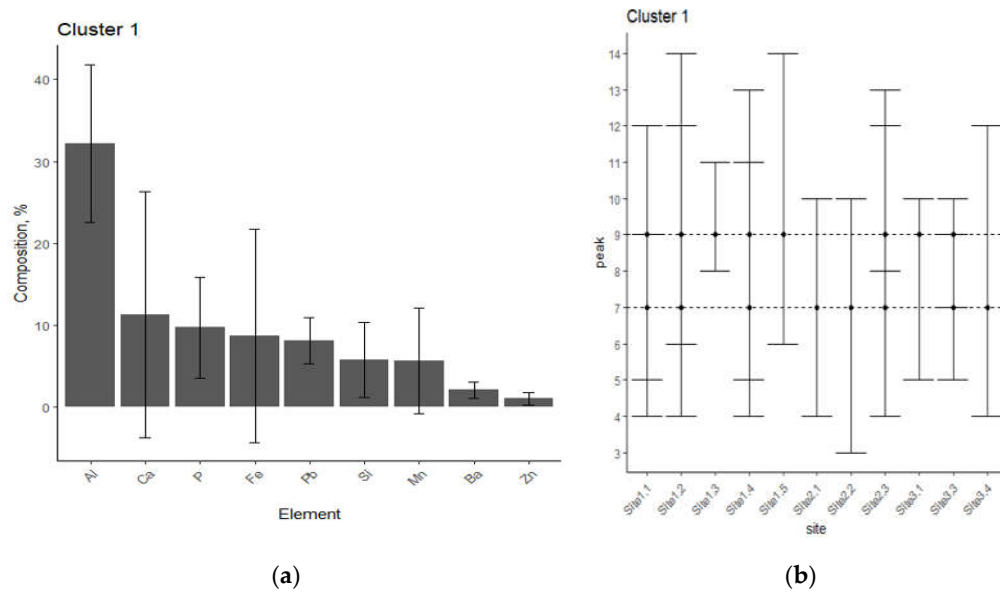
At this stage, we have grouped the individual geochemical components from the soil samples from each site into clusters and the average geochemical composition of each cluster provides a common overview of the geochemistry of the components across all soils. The other piece of information that the individual geochemical components provides is the strength of acid extract at which they are extracted. This gives a measure of the relative mobility of each geochemical component, i.e., those extracted at lower acid strength are considered more mobile than those extracted at a higher acid strength. This measure of mobility can be expressed as an extraction window defined by three pieces of information:

- i. the extraction step when the geochemical component first appears;
- ii. the extraction step where the maximum amount is extracted (i.e., the extraction peak); and
- iii. the last extraction step where the geochemical component appears.

In Figures 4–9 the left-hand plot (a) provides the mean value and standard deviation of the percentage composition of the main contributing elements for a given cluster. The smaller the standard deviation of the mean value shows that the composition of the individual geochemical components within a cluster have similar element compositions. In the right-hand plot (b), the y axis shows the extraction window for each geochemical component (the dot represents the peak value and the top and bottom whiskers represent the first and last extraction points) in each soil associated with a specific cluster. For some soils more than one of the geochemical components is associated with a single cluster and hence some soils have more than one extraction window.

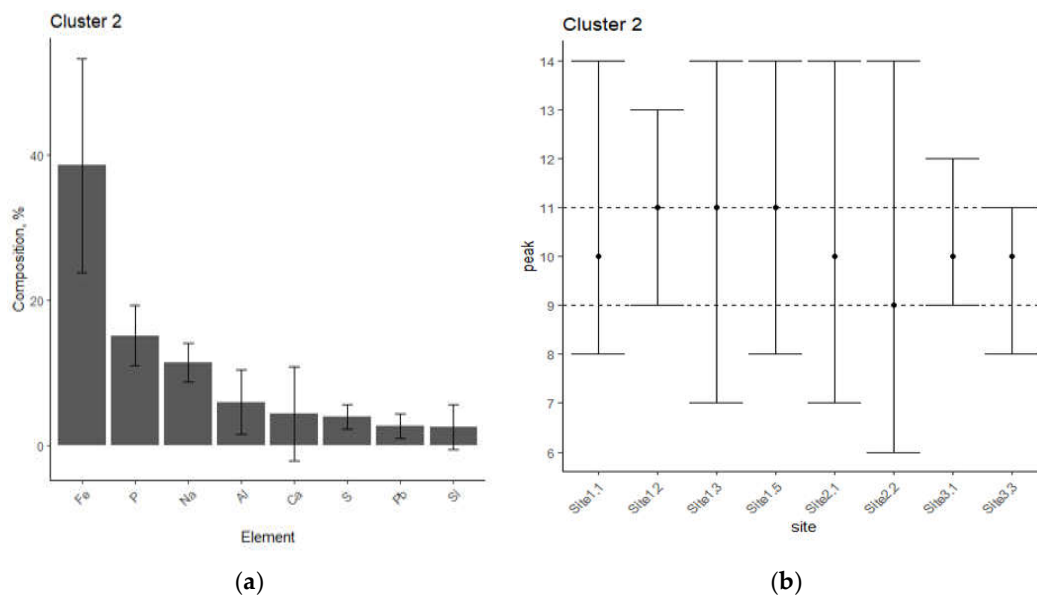
Cluster 1 is Al dominated, most likely comprising Al oxide, and from peak extraction steps 7–9 (Figure 4). The three major elements in cluster 1 with the smallest relative standard deviation are Al (ca. 30%), P (ca. 10%) and Pb (ca. 10%). Aluminium (hydr)oxides can be common in the clay fraction of soils, occurring as discrete particles, as surface coatings or as interlayers between clay mineral surfaces, and the literature attests to their ability to sorb metal ions, such as Pb, reducing their solubility [31]. The presence of an Al and P component also containing 10% Pb suggests that this component may also reflect the occurrence of a Pb-Al-phosphate mineral, such as the mineral plumbogummite ( $\text{PbAl}_3(\text{PO}_4)_2(\text{OH})_5 \cdot \text{H}_2\text{O}$ ) which has been shown to be present across a range of soil types [32–36]. In a study of contaminated soil and Pb mine waste [37] phosphate amendment was applied to determine if this addition generated a stable form of Pb. Using a combination of synchrotron-based analysis techniques, the authors showed that, after aging for 4 weeks up to 1 year, plumbogummite was formed as a stable end point. As such, it is highly probable that over the many years the Pb has been accumulating in these urban soils that the natural aging process has led to the formation of stable Pb-phosphate minerals, such as plumbogummite. However, without further investigation using scanning electron microscopy or X-ray Absorption Fine Structure Spectroscopy (XAFS), we are unable to identify which Pb-phosphate minerals are present.





**Figure 4.** (a) Average chemical composition and standard deviation of the components in cluster 1; (b) extraction peak location and their windows of extraction for cluster 1 components.

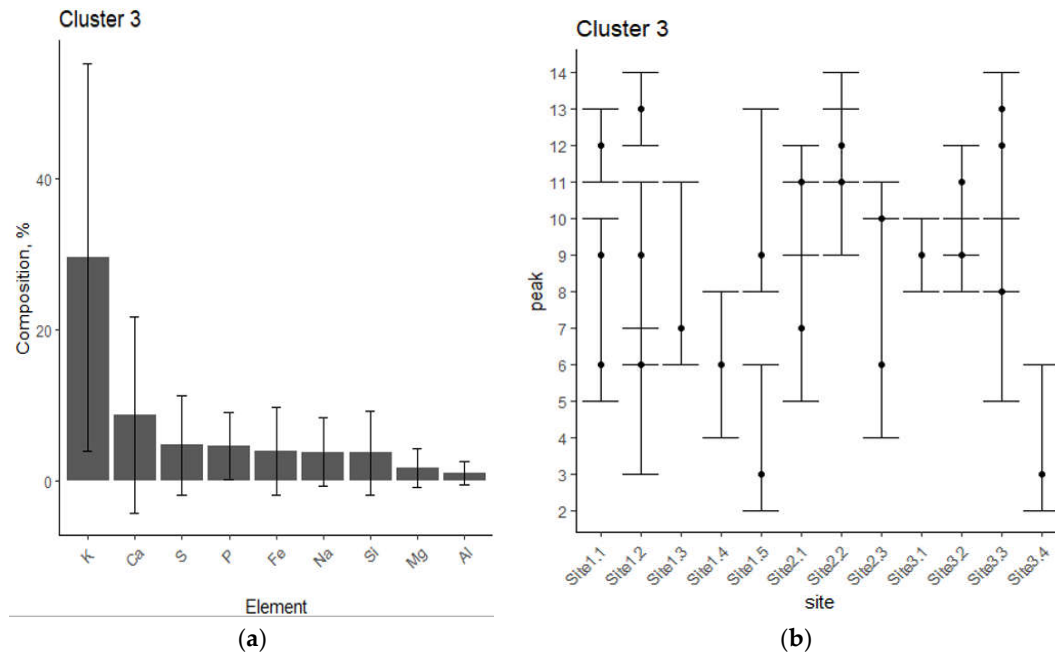
Cluster 2 contains on average 40% Fe, extracted at high acid concentration (peak extraction steps 9–11), Figure 5. Although this component has a relatively high Fe content it also contains appreciable amounts of other elements (P, Na, Al, S, Pb) suggesting that is probably an Fe oxy-hydroxide component [38].



**Figure 5.** (a) Average chemical composition and standard deviation of the components in cluster 2; (b) extraction peak location and their windows of extraction for cluster 2 components.

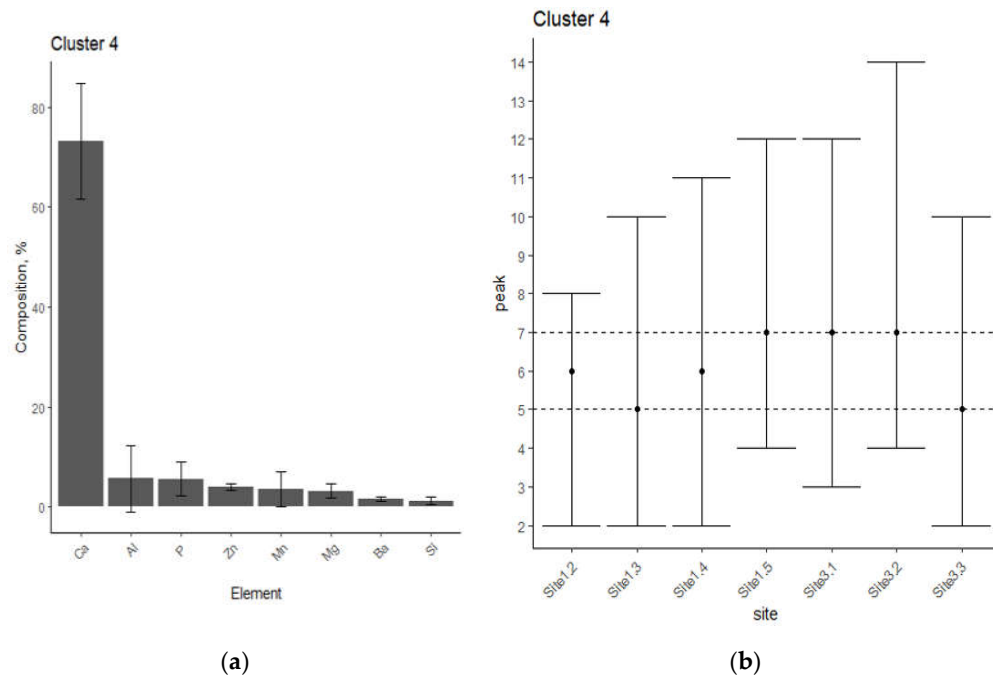
Cluster 3 has the most variable composition of all six components because it has the largest relative standard deviation in composition, but is dominated by K (Figure 6). It also has the widest range of peak extraction windows (steps 3–13). A clue to the source of this cluster comes from a study of the major element composition of sequential extracts from a variety of soils from Silesia [39], which shows that the organic fraction contains appreciable amounts of K, Ca and S but is highly variable

between the soils. This cluster is therefore possibly related to organic matter breakdown, however, the Pb content of this is low (<5%).



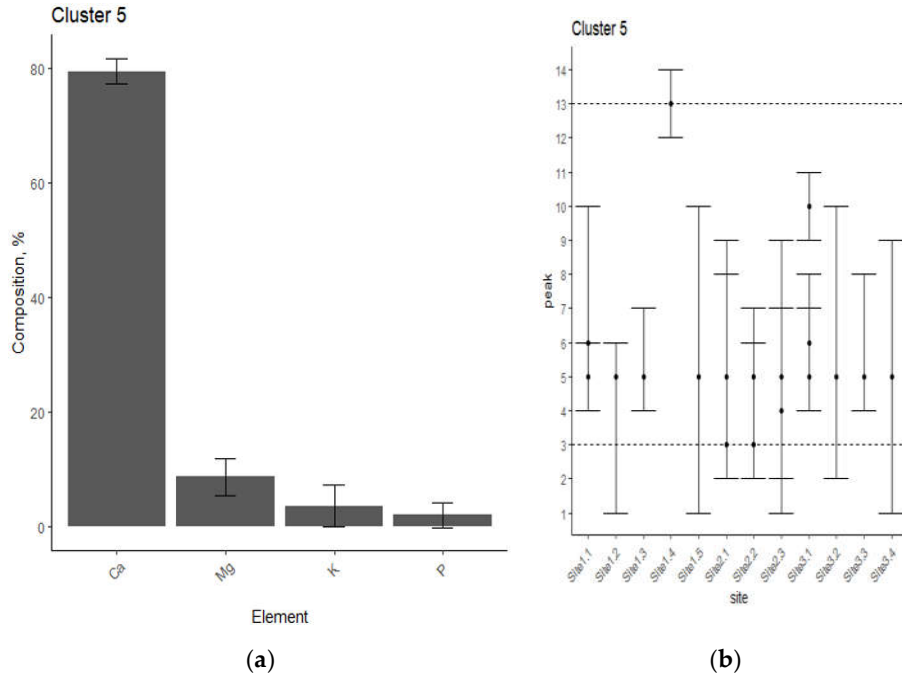
**Figure 6.** (a) Average chemical composition and standard deviation of the components in cluster 3; (b) extraction peak location and their windows of extraction for cluster 3 components.

Cluster 4 is Ca dominated (ca. 70%) and is extracted at low acid concentrations (component peaks at steps 5–7) which suggest this is a Ca carbonate component (Figure 7). The median Pb content of this cluster is below 5%.



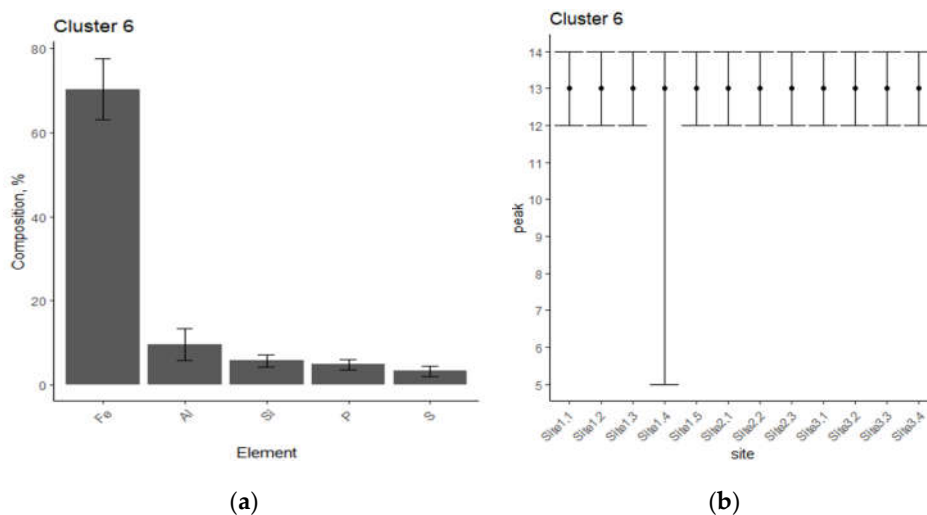
**Figure 7.** (a) Average chemical composition and standard deviation of the components in cluster 4; (b) extraction peak location and their windows of extraction for cluster 4 components.

Cluster 5 is another Ca dominated cluster (Figure 8) in which the components have a slightly higher Ca content (ca. 80%) and a much lower variability in Ca content suggesting it represents a purer form of Ca carbonate than cluster 4. The peaks of extraction of each component are more variable than those in cluster 4 (3–13). This may suggest that some of these components are more crystalline than those in cluster 4 which may be a more fine-grained amorphous material.



**Figure 8.** (a) Average chemical composition and standard deviation of the components in cluster 5; (b) extraction peak location and their windows of extraction for cluster 5 components.

Cluster 6 is a Fe dominated cluster (Figure 9) with a higher than average Fe content than that of cluster 2 and a well-defined peak of extraction of step 13 for all sites. The high acid strength and the purity of this cluster suggests it is derived from dissolution of crystalline Fe oxides [38]. The median Pb content of this cluster is less than 5%.

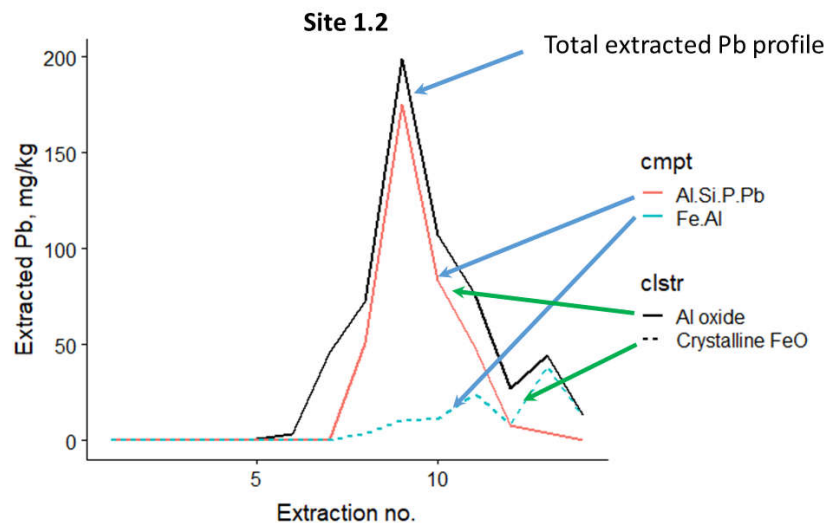


**Figure 9.** (a) Standard deviation and average chemical composition of the components in cluster 6; (b) extraction peak location and their windows of extraction for cluster 6 components.

Although the clustering process highlights the common features of the physico-chemical components of the soils studied, the individual components identified for each soil (Figure 2) and the differences between extraction windows (Figures 4–9) for different components in each soil illustrate the heterogeneity of the soils, not only between sites but also within sites. This may reflect differences in soil preparations used by each allotment holder (see SI.1).

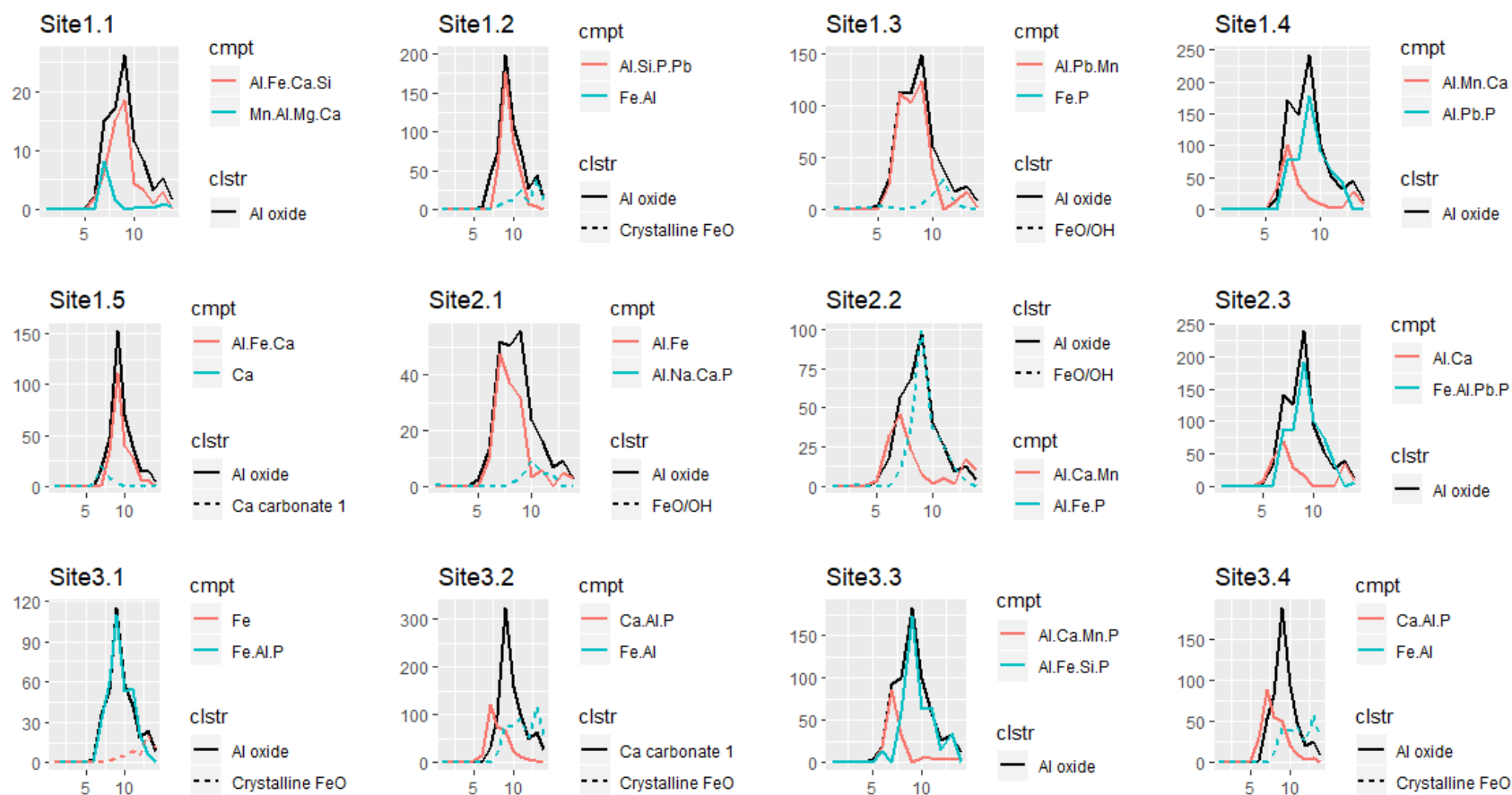
### 3.1.4. Soil Substrate Contributions to Pb Extraction Profiles

Following categorization of the geochemical components in each soil into clusters and formulation of tentative geochemical interpretations, a closer examination can be made of the contributions to the original Pb extraction profiles (Figure 1). Figure 10 shows how the two main contributing geochemical components and their associated clusters contribute to the extraction profile for the site 1.2 sample. In this instance the main contributor is the component that belongs to cluster 1, the Al oxide component, with a lesser proportion coming from the crystalline Fe oxide cluster (cluster 6). Applying the same approach to all of the samples, we can obtain an overview of the source of the main geochemical fractions contributing to the total Pb content of the soils. Figure 11 shows the two main contributing clusters for all of the soil samples under study.



**Figure 10.** Example plot of the underlying contributions to the total extracted Pb profile (solid black line). The two main contributing components (cmpt) are indicated by colour for this specific soil (red and blue line). The clusters (clstr) to which the components belong are shown by line type (dashed and solid line). In this example the solid line indicates the Al oxide cluster and dashed line indicates the crystalline FeO cluster.

Examining each profile for each soil combined with its two major contributing geochemical components, we see that in 10 of the 12 soils the most important contribution comes from the Al oxide cluster. In site 3.2, however, the most important cluster is Ca carbonate (cluster 4) and in site 3.1 it is the Fe oxyhydroxide cluster (cluster 2). The second most important cluster in all cases is either the Fe oxyhydroxide cluster (cluster 2) or the crystalline FeO cluster (cluster 6).



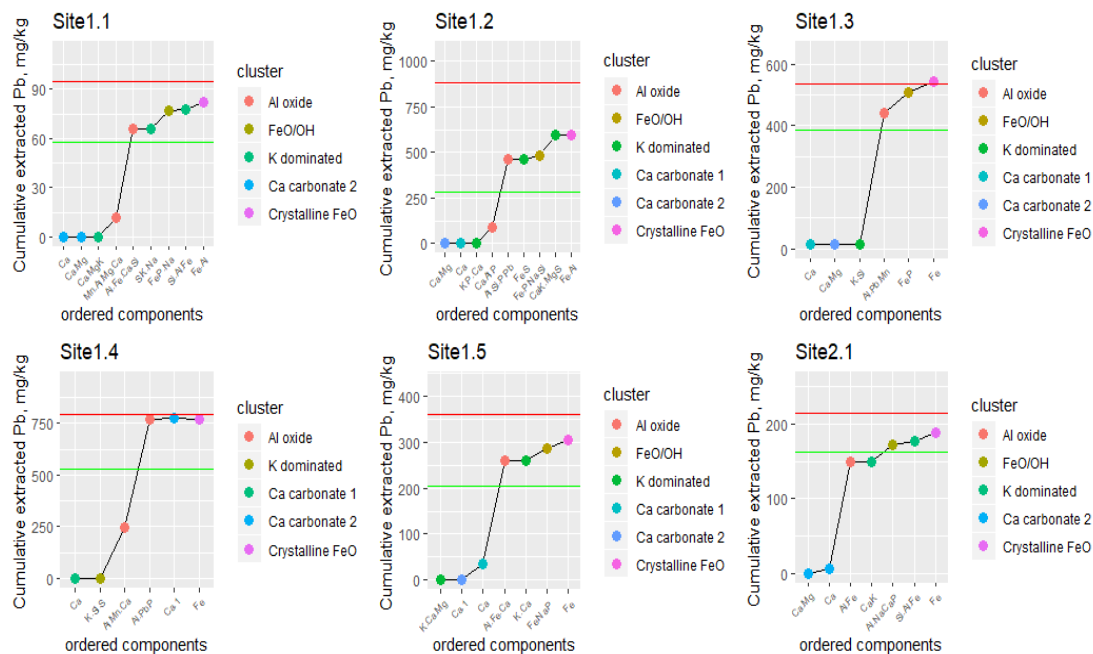
**Figure 11.** Fractionation of the CISED Pb extraction profile for all soil samples under study. “Cmpt” indicates the original component names which are shown as different colours and “clstr” indicates the clusters to which the components belong, which are shown as different line types (dashed or solid). The two components are those that make up the two main contributing sources of Pb.

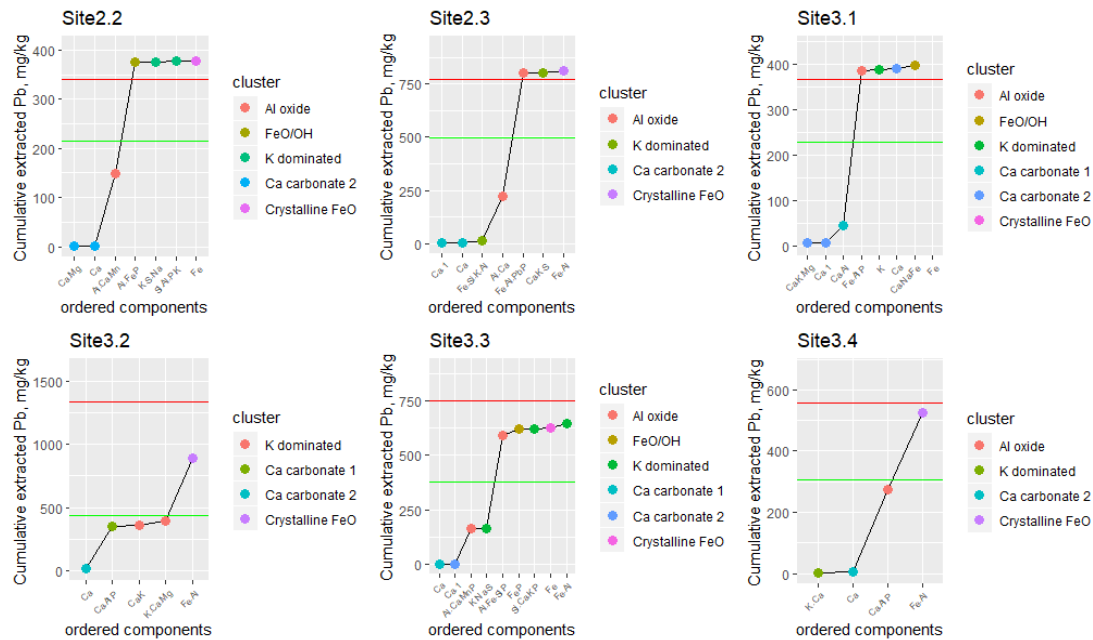
### 3.1.5. Relationship Between Pb Fractionation and Bioaccessibility

Having identified the geochemical sources of Pb in the soils through the CISED extraction process we can now compare the CISED identified fractions with the UBM bioaccessibility values and the total Pb concentrations in the soils. For each soil, the cumulative extracted Pb arising from each CISED identified component (in order of their extraction which should indicate relative availability) can be plotted and compared to the total Pb content of the soil and the UBM bioaccessibility (total and bioaccessible Pb values from Entwistle et al. [4]). The coloured points indicate the geochemical clusters that the individual soil components come from. The CISED total Pb is usually less than the whole soil digest value because Pb bound to the alumina-silicate matrix will not be extracted by the mineral acid extractants.

An examination of the cumulative plots in Figure 12 provides information on which of the soil component/clusters is responsible for the bioaccessible fraction of the soil. The clusters that contribute to the cumulative curve up to the bioaccessible value (the green horizontal line) are therefore deemed to be the sources of the bioaccessible fraction. Figure 12 shows that, in most of the samples, Pb coming from the components that make up the Al oxide cluster are an important source of the bioaccessible Pb (i.e., the Pb extracted under the green line). The Al oxide cluster, however, is made up of different components in each soil and the Figure 12 plots indicate that varying amounts of Pb in the components in this cluster contribute to the final bioaccessible Pb value. This explains why we observed no significant correlation (bootstrapped Spearman correlation confidence intervals straddle zero) of the bioaccessible Pb (either measured as an absolute value or as a percentage of the total Pb) with the Al oxide fraction.

For all of the soils, with the exception of that of site 3.2, soil components from the Al oxide cluster (cluster 1 in Figure 3) are the main contributors to the bioaccessible fraction. For the site 3.2 sample the main contributing cluster is a Ca carbonate grouping (cluster 4 in Figure 3). There is no indication from the history of chemical and soil improver usage at this sample site to explain this difference (Table SI-1).





**Figure 12.** Comparison of cumulative CISED extracted Pb and the bioaccessible and total Pb in the soils. The solid red line indicates the total Pb content and the solid green line indicates the bioaccessible Pb content.

## 4. Discussion

Lead contamination of urban soil continues to pose a potential public health threat. For widespread diffuse Pb pollution in urban areas, in situ remediation methods involving the treatment of the soil, either through the use of chemical treatments (such as addition of phosphorus containing compounds), biological remediation (such as phyto-stabilisation using ground cover) or by dilution (such as through the addition of compost and other biosolids), are gaining prominence (see reviews [40,41]). Studies on contaminated urban soils have shown Pb to be present in complexes with organic matter, phosphorus, carbonates and/or iron oxides (e.g., [42–45]). Our CISED sequential extraction data shows that the Pb in our UAS soils is primarily associated with Al oxide phases (Al dominated, Figure 3), with the second most important phase associated with either Fe oxyhydroxide or crystalline FeO, and only to a very limited extent to Ca carbonates and organic matter (Figure 11). Many studies report reduced soil mobility of Pb in the form of Pb-phosphates, such as the secondary mineral phases of the pyromorphite family [46], and 75% of the urban gardeners reported regular (at least annual) application of P-rich materials, such as compost and manure (SI-1). We interpret the co-presence of a P component with the Al oxide cluster to suggest the soils contain Pb phosphate type minerals, possibly the mineral plumbogummite ( $\text{PbAl}_3(\text{PO}_4)_2(\text{OH})_5 \cdot \text{H}_2\text{O}$ ). Indeed, the formation of naturally occurring less-labile and non-labile (or fixed) forms of Pb over time, as a consequence of natural time-dependent (“soil aging”) processes, is well reported in the literature [47].

In our study, the Pb associated with the Al oxide–P phase appears to be the major source of the UBM bioaccessible fraction of Pb, with smaller associations of Pb with Fe oxide and carbonate phases (Figure 12). There is some suggestion that *in vitro* tests overestimate the bioaccessible fraction when phosphate is co-present. A recent study on highly contaminated soil (1500–8000 mg/kg Pb) by smelting and mining activity showed that the UBM overestimated bioaccessibility in some of the soils [48] compared to a mouse animal model. Another study [49] showed that uncertainties in comparing *in vitro* testing to an animal mouse model to investigate the effect of phosphate amendments on Pb bioaccessibility makes it difficult to confirm overestimation of *in vitro* tests. The form of Pb in soil in particular is influenced by the source, absolute concentration of Pb, chemical composition of the soil and aging, but it is clear that insoluble Pb-phosphates are formed in the presence of phosphate

amendments [46]. The degree to which these reduce bioaccessibility/bioavailability is still to be determined, particularly in urban agricultural soils. A recent review of the use of phosphate compounds to remediate urban Pb contaminated soils highlighted the need for caution because current understanding is principally drawn from tests on highly contaminated soils (typically from firing ranges and mine wastes), using high P dosing rates, over experimental periods typically limited to less than 24 months [40]. Addition of phosphate amendments can also have some negative side effects, which include the risk of primary P leaching and eutrophication of surface water sources, and the possibility of As enhanced leaching [46].

On the basis of our CISED data, in tandem with our biomonitoring data, which indicates the lack of elevated blood Pb levels in our gardeners compared to their non-gardening neighbours, we conclude the (legacy) Pb in these soils has been rendered relatively immobile. However, the lack of a statistically significant difference in BLL between the two groups might feasibly be related to differences in exposure, rather than solely a product of low soil Pb mobility. To account for this, participant gardeners recruited a neighbour or friend of the same sex and similar age to act as their control. Thus, the control population was as close a match to the gardeners as possible; they were their close neighbours, so were subject to similar ambient Pb exposure, and lived in similar housing stock. To account for confounding variables in our statistical modelling, all participants provided information on personal characteristics including age, sex, alcohol consumption, smoking, occupations and hobbies that may lead to Pb exposure, and domestic cleaning habits, in addition to building age, whether they had lead pipes for tap water, and whether they kept cats or dogs as pets [50]. Gardener participants also provided information on frequency and duration of visits to the UAS, and rates of consumption of fruit and vegetables (both shop-bought and homegrown). The majority of gardeners in our study visited their garden several times a week (during spring, summer and autumn), typically for 2–4 h, and the consumption rate data indicated a high percentage of homegrown fruit (herbaceous and shrub) and vegetable (green, root and tuber) consumption in the diet (ranging from 21% to 54% for 50th percentile consumers) [4]. Our exposure indicators thus suggested a potentially higher exposure to Pb for the gardeners compared to our controls. The observed lack of any significant difference in the two cohorts' BLL supports our interpretation that the Pb in these soils has been rendered relatively immobile.

Our findings suggest that expensive barrier and “fixation” treatments do not appear to be justified in these urban allotments. The advice to our urban gardeners, based on our findings, is to follow good land management and hygiene practices such as: removing outdoor footwear before entering the home and storing gardening equipment outside, or in a designated area near the doorway, to reduce the back-tracking of soil into the home; thoroughly washing soil-laden crops outside; peeling crops where feasible before eating; keeping soil moist (or covered) during dry periods and in windy conditions; and diluting the Pb concentration of the soil using sustainable organic-rich amendments such as animal manure composts, biosolids, green-waste and biochar [51–53].

## 5. Conclusions

Our study aimed to understand the geochemical controls on the bioaccessibility of Pb in soils from urban allotment sites. This knowledge has been used as part of an approach involving multiple lines of evidence to assess the hazards present in allotment sites, and inform land management and future use strategies. We have shown that the Pb in our urban soils is primarily associated with Al oxide phases, with the second most important phase associated with either Fe oxyhydroxide or crystalline FeO, and only to a limited extent with Ca carbonates. The CISED method has been shown to provide additional information on element fraction in addition to that of classical sequential extraction methods [27,28]. The co-presence of a P component with the Al oxide cluster indicates the soils contain Pb phosphate type minerals, likely as a by-product of natural “soil aging” processes. This information provides an indication of the chemical forms of Pb in the UAS and their potential lability and bioaccessibility. Indeed, the presence of Pb phosphates, in conjunction with our biomonitoring data which shows the lack of elevated blood Pb levels in our gardeners, indicates the (legacy) Pb in these soils is relatively immobile. This study has given confidence to the local authority regulators and the gardeners that these



urban gardens can be safe to use, even where soil Pb levels are up to ten times above the UK's recommended lead screening level. Without this evidence, closure and redevelopment of such sites were a real concern. The work does highlight some heterogeneity between individual allotment plots, both within sites and across multiple locations, and serves as a reminder that there may not be a "one size fits all" approach to remediation/re-use.

Although the debate continues regarding the efficacy of using P-rich amendments to reduce the bioaccessibility/bioavailability of soil Pb, the often poor quality of many urban soils, or the increasing desire to utilize raised beds for more accessible gardening, can be seen as a positive driver of Pb dilution and P additions over the years. Methods for intervention/remediation need to be low cost, and utilise locally available materials and/or readily implementable practices, to facilitate widespread adoption. Employment of good hygiene practices remains important for all visitors to UAS, not just the urban gardeners. Projects that facilitate wider public understanding of environmental pollution and everyday exposures, such as the academic–regulator–community partnership presented here, have an important role to play in raising awareness of environmental health issues associated with both legacy and new generation pollutants. Indeed, increased hand-washing and exposure awareness linked to the Covid-19 pandemic may yet prove to be a positive driver of reduced exposure to contaminants, such as Pb, in urban settings.

**Supplementary Materials:** The following is available online at [www.mdpi.com/2076-3263/10/10/398/s1](http://www.mdpi.com/2076-3263/10/10/398/s1), S1: Background information on use of soil improvers and chemicals on allotments included in the NABS study.

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## References

1. Van Den Berg, A.E.; Custers, M.H. Gardening promotes neuroendocrine and affective restoration from stress. *J. Health Psychol.* **2011**, *16*, 3–11.
2. Soga, M.; Gaston, K.J.; Yamaura, Y. Gardening is beneficial for health: A meta-analysis. *Prev. Med. Rep.* **2017**, *5*, 92–99.
3. Rouillon, M.; Harvey, P.J.; Kristensen, L.J.; George, S.G.; Taylor, M.P. VegeSafe: A community science program measuring soil-metal contamination, evaluating risk and providing advice for safe gardening. *Environ. Pollut.* **2017**, *222*, 557–566.
4. Entwistle, J.A.; Amaibi, P.M.; Dean, J.R.; Deary, M.E.; Medock, D.; Morton, J.; Rodushkin, I.; Bramwell, L. An apple a day? Assessing gardeners' lead exposure in urban agriculture sites to improve the derivation of soil assessment criteria. *Environ. Int.* **2019**, *122*, 130–141, doi:10.1016/j.envint.2018.10.054.
5. Lanphear, B.P.; Rauch, S.; Auinger, P.; Allen, R.W.; Hornung, R.W. Low-level lead exposure and mortality in US adults: A population-based cohort study. *Lancet Public Health* **2018**, *3*, e177–e184.
6. Boisa, N.; Bird, G.; Brewer, P.; Dean, J.; Entwistle, J.A.; Kemp, S.; Macklin, M. Potentially harmful elements (PHEs) in scalp hair, soil and metallurgical wastes in Mitrovica, Kosovo: The role of oral bioaccessibility and mineralogy in human PHE exposure. *Environ. Int.* **2013**, *60*, 56–70.
7. Office for National Statistics, National Records of Scotland, Northern Ireland Statistics and Research Agency. (2016). In *2011 Census Aggregate Data*; UK Data Service (Edition: June 2016); Office for National Statistics: London, UK, 2016; doi:10.5257/census/aggregate-2011-1.

8. Walkley, A.; Black, I.A. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci.* **1934**, *37*, 29–38.
9. BARGE. Bioaccessibility Research Group of Europe. Available online: <http://www.bgs.ac.uk/barge/home.html> (accessed on 4th October, 2020).
10. Denys, S.; Caboche, J.; Tack, K.; Rychen, G.; Wragg, J.; Cave, M.; Jondreville, C.; Feidt, C. In vivo validation of the unified BARGE method to assess the bioaccessibility of arsenic, antimony, cadmium, and lead in soils. *Environ. Sci. Technol.* **2012**, *46*, 6252–6260, doi:10.1021/es3006942.
11. ISO. Assessment of human exposure from ingestion of soil and soil material -- Procedure for the estimation of the human bioaccessibility/bioavailability of metals in soil. ISO 17924:2018.
12. Amaibi, P.M.; Entwistle, J.A.; Kennedy, N.; Cave, M.; Kemp, S.; Potgieter-Vermaak, S.; Dean, J.R. Mineralogy, solid-phase fractionation and chemical extraction to assess the mobility and availability of arsenic in an urban environment. *Applied Geochemistry*. **2018**, *100*, 244–257 doi:10.1016/j.apgeochem.2018.12.004.
13. Giacomino, A.; Abollino, O.; Malandrino, M.; Mentasti, E. The role of chemometrics in single and sequential extraction assays: A Review. Part II. Cluster analysis, multiple linear regression, mixture resolution, experimental design and other techniques. *Anal. Chim. Acta* **2011**, *688*, 122–139, doi:10.1016/j.aca.2010.12.028.
14. Wragg, J.; Cave, M.; Gregory, S. The solid phase distribution and bioaccessibility of arsenic, chromium, and nickel in natural ironstone soils in the UK. *Appl. Environ. Soil Sci.* **2014**, Article ID 924891.
15. Wragg, J.; Cave, M.R. Assessment of a geochemical extraction procedure to determine the solid phase fractionation and bioaccessibility of potentially harmful elements in soils: A case study using the NIST 2710 reference soil. *Anal. Chim. Acta* **2012**, *722*, 43–54, doi:10.1016/j.aca.2012.02.008.
16. Cave, M. *The Use of Self Modelling Mixture Resolution Methods for the Interpretation of Geochemical Data Sets* IR/08/035; British Geological Survey: Keyworth, Nottingham, UK, 2008.
17. Filgueiras, A.; Lavilla, I.; Bendicho, C. Chemical sequential extraction for metal partitioning in environmental solid samples. *J. Environ. Monit.* **2002**, *4*, 823–857.
18. Mackey, E.; Christopher, S.; Lindstrom, R.; Long, S.; Marlow, A.; Murphy, K.; Paul, R.; Popelka-Filcoff, R.; Rabb, S.; Sieber, J. Certification of three NIST renewal soil standard reference materials for element content: SRM 2709a San Joaquin Soil, SRM 2710a Montana Soil I, and SRM 2711a Montana Soil II. *Nist Spec. Publ.* **2010**, *260*, 1–39.
19. Sutherland, R.A. BCR®-701: A review of 10-years of sequential extraction analyses. *Anal. Chim. Acta* **2010**, *680*, 10–20, doi:10.1016/j.aca.2010.09.016.
20. Wragg, J. *BGS Guidance Material 102, Ironstone Soil, Certificate of Analysis*; IR/09/006; British Geological Survey: Keyworth, Nottingham, UK, 2009.
21. Cave, M.R.; Milodowski, A.E.; Friel, E.N. Evaluation of a method for identification of host physico-chemical phases for trace metals and measurement of their solid-phase partitioning in soil samples by nitric acid extraction and chemometric mixture resolution. *Geochem. Explor. Environ. Anal.* **2004**, *4*, 71–86, doi:10.1144/1467-7873/03-025.
22. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2019.
23. Cox, S.F.; Rollinson, G.; McKinley, J.M. Mineralogical characterisation to improve understanding of oral bioaccessibility of Cr and Ni in basaltic soils in Northern Ireland. *J. Geochem. Explor.* **2017**, *183*, 166–177, doi:10.1016/j.gexplo.2017.02.006.
24. Gal, J.; Hursthouse, A.; Cuthbert, S. Bioavailability of arsenic and antimony in soils from an abandoned mining area, Glendinning (SW Scotland). *J. Environ. Sci. Health Part A* **2007**, *42*, 1263–1274, doi:10.1080/10934520701435585.
25. Tardif, S.; Cipullo, S.; So, H.U.; Wragg, J.; Holm, P.E.; Coulon, F.; Brandt, K.K.; Cave, M. Factors governing the solid phase distribution of Cr, Cu and As in contaminated soil after 40 years of ageing. *Sci. Total Environ.* **2019**, *652*, 744–754, doi:10.1016/j.scitotenv.2018.10.244.
26. Wragg, J.; Broadway, A.; Cave, M.R.; Fordyce, F.M.; Palumbo-Roe, B.; Beriro, D.J.; Farmer, J.G.; Graham, M.C.; Ngwenya, B.T.; Bewley, R.J.F. Linkage between solid-phase apportionment and bioaccessible arsenic, chromium and lead in soil from Glasgow, Scotland, UK. *Earth Environ. Sci. Trans. R. Soc. Edinb.* **2019**, *108*, 217–230, doi:10.1017/s1755691018000762.

27. Rauret, G.; F. López-Sánchez, J.; Sahuquillo, A.; Rubio, R.; Davidson, C.; Ure, A.; Quevauviller, P. Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. *J. Environ. Monit.* **1999**, *1*, 57–61, doi:10.1039/A807854H.
28. Tessier, A.; Campbell, P.G.C.; Bisson, M. Sequential extraction procedure for the speciation of particulate trace metals. *Anal. Chem.* **1979**, *51*, 844–851, doi:10.1021/ac50043a017.
29. Reis, A.P.; Patinha, C.; Wragg, J.; Dias, A.C.; Cave, M.; Sousa, A.J.; Costa, C.; Cachada, A.; Ferreira da Silva, E.; Rocha, F.; et al. Geochemistry, mineralogy, solid-phase fractionation and oral bioaccessibility of lead in urban soils of Lisbon. *Environ. Geochem. Health* **2014**, *36*, 867–881.
30. Fraley, C.; Raftery, A.E. Model-based methods of classification: Using the mclust software in chemometrics. *J. Stat. Softw.* **2007**, *18*, 1–13.
31. Zhuang, J.; Yu, G.R. Effects of surface coatings on electrochemical properties and contaminant sorption of clay minerals. *Chemosphere* **2002**, *49*, 619–628, doi:10.1016/s0045-6535(02)00332-6.
32. Adams, J.A.; Howarth, D.T.; Campbell, A.S. Plumbogummite minerals in a strongly weathered New Zealand soil. *J. Soil Sci.* **1973**, *24*, 224–231.
33. Ildefonse, P.; Morin, G.; Juillot, F.; Calas, G.; Samama, J.C.; Brown, G.E.; Chevallier, P.; Populus, P.; Weathering of a Pb-mineralized sandstone, Ardeche, France: Lead speciation. In *Geochemistry of the Earth's Surface*; Armannsson, H., Ed.; Balema: Rotterdam, The Netherlands, 1999; pp. 385–388.
34. Morin, G.; Juillot, F.; Ildefonse, P.; Calas, G.; Samama, J.C.; Chevallier, P.; Brown, G.E. Mineralogy of lead in a soil developed on a Pb-mineralized sandstone (Largentiere, France). *Am. Mineral.* **2001**, *86*, 92–104.
35. Nriagu, J.O. Formation and stability of base metal phosphates in soils and sediments. In *Phosphate Minerals*; Springer: Berlin/Heidelberg, Germany, 1984; pp. 318–329.
36. Sarma, V.A.K.; Krishnam, G.S. Plumbogummite minerals in Indian soils. *Geoderma* **1970**, *3*, 321, doi:10.1016/0016-7061(70)90012-1.
37. Baker, L.R.; Pierzynski, G.M.; Hettiarachchi, G.M.; Scheckel, K.G.; Newville, M. Micro-X-Ray Fluorescence, Micro-X-Ray Absorption Spectroscopy, and Micro-X-Ray Diffraction Investigation of Lead Speciation after the Addition of Different Phosphorus Amendments to a Smelter-Contaminated Soil. *J. Environ. Qual.* **2014**, *43*, 488–497, doi:10.2134/jeq2013.07.0281.
38. Cornell, R.M.; Schwertmann, U. *The Iron Oxides-Structure Properties, Reactions, Occurrences and Uses*; VCH Publishers: Weinheim, Germany, 1996.
39. Baranowski, R.; Rybak, A.; Baranowska, I. Speciation analysis of elements in soil samples by XRF. *Pol. J. Environ. Stud.* **2002**, *11*, 473–482.
40. Henry, H.; Naujokas, M.F.; Attanayake, C.; Basta, N.T.; Cheng, Z.; Hettiarachchi, G.M.; Maddaloni, M.; Schadt, C.; Scheckel, K.G. Bioavailability-Based In Situ Remediation To Meet Future Lead (Pb) Standards in Urban Soils and Gardens. *Environ. Sci. Technol.* **2015**, *49*, 8948–8958.
41. Laidlaw, M.A.; Filippelli, G.M.; Brown, S.; Paz-Ferreiro, J.; Reichman, S.M.; Netherway, P.; Truskewycz, A.; Ball, A.S.; Mielke, H.W. Case studies and evidence-based approaches to addressing urban soil lead contamination. *Appl. Geochem.* **2017**, *83*, 14–30.
42. Attanayake, C.P.; Hettiarachchi, G.M.; Harms, A.; Presley, D.; Martin, S.; Pierzynski, G.M. Field evaluations on soil plant transfer of lead from an urban garden soil. *J. Environ. Qual.* **2014**, *43*, 475–487.
43. Attanayake, C.P.; Hettiarachchi, G.M.; Martin, S.; Pierzynski, G.M. Potential bioavailability of lead, arsenic, and polycyclic aromatic hydrocarbons in compost-amended urban soils. *J. Environ. Qual.* **2015**, *44*, 930–944.
44. Cheng, Z.; Lee, L.; Dayan, S.; Grinshtein, M.; Shaw, R. Speciation of heavy metals in garden soils: Evidences from selective and sequential chemical leaching. *J. Soils Sediments* **2011**, *11*, 628.
45. Hettiarachchi, G.M.; Pierzynski, G.M.; Ransom, M.D. In situ stabilization of soil lead using phosphorus and manganese oxide. *Environ. Sci. Technol.* **2000**, *34*, 4614–4619.
46. Miretzky, P.; Fernandez-Cirelli, A. Phosphates for Pb immobilization in soils: A review. *Environ. Chem. Lett.* **2008**, *6*, 121–133.
47. Villen-Guzman, M.; Garcia-Rubio, A.; Paz-Garcia, J.M.; Vereda-Alonso, C.; Gomez-Lahoz, C.; Rodriguez-Maroto, J.M. Aging effects on the mobility of Pb in soil: Influence on the energy requirements in electroremediation. *Chemosphere* **2018**, *213*, 351–357.
48. Li, S.-W.; Liu, X.; Sun, H.-J.; Li, M.-Y.; Zhao, D.; Luo, J.; Li, H.-B.; Ma, L.Q. Effect of phosphate amendment on relative bioavailability and bioaccessibility of lead and arsenic in contaminated soils. *J. Hazard. Mater.* **2017**, *339*, 256–263.

49. Kastury, F.; Placitu, S.; Boland, J.; Karna, R.R.; Scheckel, K.G.; Smith, E.; Juhasz, A.L. Relationship between Pb relative bioavailability and bioaccessibility in phosphate amended soil: Uncertainty associated with predicting Pb immobilization efficacy using in vitro assays. *Environ. Int.* **2019**, *131*, 104967, doi:10.1016/j.envint.2019.104967.
50. Bramwell, L.; Morton, J.; Harding, A.-H.; Lin, N.; Entwistle, J. Determinants of adult lead body burdens including exposures from urban agricultural sites. (Unpublished report on the Newcastle Allotments Biomonitoring Study [NABS]).
51. Brown, S.; Chaney, R.L.; Hallfrisch, J.G.; Xue, Q. Effect of biosolids processing on lead bioavailability in an urban soil. *J. Environ. Qual.* **2003**, *32*, 100–108, doi:10.2134/jeq2003.1000.
52. Brown, S.L.; Chaney, R.L.; Hettiarachchi, G.M. Lead in Urban Soils: A Real or Perceived Concern for Urban Agriculture? *J. Environ. Qual.* **2016**, *45*, 26–36, doi:10.2134/jeq2015.07.0376.
53. Paz-Ferreiro, J.; Lu, H.; Fu, S.; Méndez, A.; Gascó, G. Use of phytoremediation and biochar to remediate heavy metal polluted soils: A review. *Solid Earth* **2014**, *5*, 65–75, doi:10.5194/se-5-65-2014.



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